



## IN-VITRO ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL OF LEAF AND FLOWER EXTRACTS OF *VERNONIA CINEREA* AND THEIR PHYTOCHEMICAL CONSTITUENTS

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### ABSTRACT

In the present study preliminary phytochemical analysis of methanol extract of leaf and flower indicated the presence of alkaloids, phenols, tannin, saponins and flavonoids. The antibacterial activity of different extracts (hexane, petroleum ether, chloroform and methanol) of leaf and flower of *Vernonia cinerea* were tested separately against both gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram negative (*Enterobacter aerogenes*) bacteria using the agar well diffusion method. Extracts of both the parts (leaf and flower) exhibited differential effects on the test bacteria. Maximum antibacterial activity (14 mm) was noticed with the methanolic extract of leaf followed by hexane extract (13 mm) of flower against *S. aureus* and *B. cereus*, respectively. The antibacterial potential of the methanol extract of leaf against the test bacteria determined by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) indicated the higher susceptibility of the *B. cereus* and *S. aureus* as compared to the *E. aerogenes*. Maximum antioxidant potential using DPPH radical scavenging capacity was noticed in methanol leaf extract. From the study it can be concluded that the antibacterial and antioxidant activities exhibited by *Vernonia cinerea* is of great interest and may lead to the discovery of new pharmacologically important compounds.

**Keywords:** Antibacterial, Antioxidant, Asteraceae, Phytochemicals, *Vernonia cinerea*.

### INTRODUCTION

Resistance of microorganisms to antibiotics has become a global issue.<sup>1</sup> Emergence of new multidrug-resistant microorganisms has reduced the efficacy of many well known antibiotics.<sup>2</sup> There is an urgent need to discover new antimicrobial compounds with novel mechanisms of action and diverse chemical structures for new and re-emerging infectious diseases.<sup>3</sup> Therefore, researchers are looking for new compounds to develop better antimicrobial drugs and focused their attention towards various natural sources like fungi, algae, and higher plants.<sup>4</sup> Among them, higher plants play an important role by producing large number of secondary metabolites which can act as chemotherapeutic, bactericidal, and bacteriostatic agents.<sup>5</sup> Plant based antimicrobials have been proved to be effective in the treatment of infectious diseases simultaneously with lesser side effects, which are often associated with synthetic antibiotics.<sup>6</sup>

The Asteraceae family is well distributed in Indian flora, by its floral structure and chemical composition; it is considered one of the most advanced family from all the Dicotyledonous.<sup>7</sup> A large number of plants belonging to the Asteraceae family contained chemical compounds exhibiting antimicrobial and antioxidant properties. Various studies have been carried out on some of Asteraceae plants, e.g. *Achillea* sp.<sup>8</sup>, *Ageratum* sp.<sup>9</sup> and *Vernonia* sp.<sup>10</sup>. The different parts of *Tridax procumbens* were also evaluated for antibacterial and antifungal activity against *E. coli*, *S. aureus*, *P. mirabilis* and *C. albicans*.<sup>11</sup> Candan et al<sup>8</sup> has reported the antimicrobial and antioxidant potential of *Achillea* species of family Asteraceae. Traditionally, *Ageratum* sp. is used as

antidysenteric<sup>9</sup> and in the treatment of leprosy and purulent ophthalmia.<sup>12</sup> Roots and leaves of *Vernonia* sp. are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort.<sup>10</sup> Furthermore, literature survey revealed that little attention has been given to antibacterial and antioxidant potential of different extracts of aerial parts of the *Vernonia* sp.

Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and gram negative bacteria (*Enterobacter aerogenes*) were used as a test bacteria as it has been shown to be major causal organisms of various human infections such as food poisoning, nosocomial infections, wound infections and urinary tract infections. Therefore, the aim of this study was to analyze the phytochemical constituents, antibacterial and antioxidant properties of leaf and flower of *Vernonia cinerea*.

### MATERIALS AND METHODS

#### Plant material

The leaves and flowers of *Vernonia cinerea* were collected locally from kukrail forest, lucknow, India on 29<sup>th</sup> December 2009. The authenticity of the plant was confirmed by Dr. Tariq Husain, Scientist, NBRI and voucher specimens are maintained at the NBRI herbarium.

#### Preparation of plant extracts

The dust free leaves and flowers of *Vernonia cinerea* were shade dried for five days. The plant material were finely ground and 25 g of dried powder from each part were then extracted sequentially by using soxhlet extractor



with 250 ml of hexane, petroleum ether, chloroform and methanol separately in order to extract non-polar and polar compounds.<sup>13</sup> The crude extracts were then filtered through Whatman No. 1 filter paper and concentrated in vacuum at 40°C using a rotary evaporator. The concentrated extracts were subsequently dried aseptically with the help of drier.

### Phytochemical analysis

Phytochemical analysis for major secondary metabolites from leaves and flower of *Vernonia cinerea* were undertaken using standard qualitative and quantitative methods as described by various authors.<sup>14-17</sup> The leaves and flower were screened for the presence of compounds like alkaloids, tannins, saponins, flavonoids and Phenols.

### Microorganisms and Growth conditions

*Bacillus cereus*, *Enterobacter aerogenes* and *Staphylococcus aureus* were used as test bacteria in the present study. The bacterial strains were procured from NCL pune and revived in Nutrient Broth. For antibacterial testing fresh inoculum was prepared for each bacterium and incubated at 37±2°C for 24 h. The cells suspension was adjusted with nutrient broth to obtain turbidity comparable to that of McFarland 0.5 standard ( $1.5 \times 10^8$  CFU/ml).

### Antibiotic sensitivity testing

For antibiotic sensitivity of bacterial strains against two antibiotics such as Amoxicillin and Erythromycin having potency of 10 µg per disc was determined by the standard disc diffusion method of Bauer et al.<sup>18</sup>

### Determination of antibacterial susceptibility

#### (a) Agar well diffusion method

The antibacterial assay of the extracts of leaves and flower of *Vernonia cinerea* were carried out by using agar well diffusion method.<sup>19</sup> 100 µl of diluted inoculum ( $1.5 \times 10^8$  CFU/ml) of test bacteria was swabbed over plates containing sterile Mueller Hinton agar (pH 7.2). Forty microlitre (40 µl) of the extract from various concentration prepared in DMSO (50 mg extract/ml of DMSO) was poured into the well of 4 mm diameter of agar plate. DMSO without extract was used as a control.

#### (b) Micro dilution method

Minimum inhibitory concentration (MIC) was determined by using micro broth-dilution bioassay of only selected extracts having potential antibacterial activity.<sup>20</sup> Crude plant extracts were dissolved in DMSO to make stock solution of 100 mg/ml. 100 µl of each extract stock solution were two fold serially diluted with sterile nutrient broth in a 96-wells of microtiter plates for each bacteria. Thereafter, 100 µl inoculum ( $1.5 \times 10^8$  CFU / ml bacterial suspension) was added to each well. The microtiter plates were incubated at 37±2°C for 24 h. Fifty microlitre (50 µl) of 2 mg/ml p-iodo-nitrotetrazoleum chloride (INT) to each well was added and incubated at 37°C for 30 minutes. The reddish-pink colour indicates

growth of bacteria in the microtiter plate and clear wells indicates the inhibition by extract. The MIC values were taken as the lowest concentration of extract in the well that showed no color. The minimum bactericidal concentration (MBC) was determined by subculturing 50 µl from each well showing no apparent colour and the least concentration of extract showing no visible growth on agar plate was taken as MBC.

### Determination of free radical scavenging activity

The DPPH radical scavenging capacity of methanol extracts of leaf and flower was determined following the method of Williams et al.<sup>21</sup> modified by Miliuskas et al.<sup>22</sup>. DPPH radicals have strong absorption maximum at 515 nm which decreases due to the reduction by antioxidants. The DPPH• solution in methanol ( $6 \times 10^{-5}$  M) was prepared freshly, and 3 mL of this solution was mixed with 100 µl of methanolic extracts. The samples were incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 515 nm was measured (AE). A blank sample containing 100 µl of methanol in the DPPH• solution was prepared, and its absorbance was measured (AB). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(AB-AE)/AB] \times 100$$

where AB = absorbance of the blank sample, and AE = absorbance of the methanol extract.

### Statistical analysis

All data are mean ± S.E. of three replicates. Statistical analysis was performed using spss version 10.

## RESULTS AND DISCUSSION

Secondary metabolites in various plants and microorganisms are generally evolved in self defense to avert the toxicity due to adverse environmental/physiological conditions. Subsequently these intermediary metabolites also provided a natural platform for development of numerous drug leads for treatment of various diseases. The present study revealed the presence of tannin, alkaloids, phenols and flavonoids in the methanol extracts of leaf and flower of *Vernonia cinerea* (Table 1).

The leaf extract showed abundant occurrence of flavonoids while in flower extracts flavonoids and saponins were predominant. The distribution and localization of these metabolites varies with the parts of the plant used which may be responsible for the differences in the antimicrobial activity.<sup>23</sup>

Figures 1a, b and c showed the effect of various concentrations (0.25, 0.5, 1 and 2 mg / well) of hexane extracts of leaf and flower on *B. cereus*, *E. aerogenes* and *S. aureus*. Extraction procedure from hexane is an important part in the isolation process of bioactive compounds since non polar compounds were extracted rapidly in hexane.<sup>24</sup> Maximum zones of inhibition of 13



mm, 11 mm and 9 mm were noticed with hexane extract of flower (2 mg /well) against *B. cereus*, *E. aerogenes* and *S. aureus*, respectively. However, hexane leaf extract at the same concentration caused inhibition zone of only 6 mm and 7 mm against *B. cereus* and *E. aerogenes*, respectively. The hexane extract of leaf did not show any activity against *S. aureus* at any concentrations. Mann et al<sup>25</sup> investigated the potent antimicrobial activity of hexane extracts of *Bombax buonopozense* (Bombacaceae) flower against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger*.

Further, the ether extracts of leaf and flower showed antibacterial activity against *B. cereus*, *E. aerogenes* and *S. aureus* (Figure 2a, b and c). Both leaf and flower extracts of *Vernonia cinerea* at high concentration of the crude extracts (2 mg/well) showed similar effects as observed by inhibitory zone (8 mm) against *B. cereus*. Whereas, *E. aerogenes* and *S. aureus* showed differential sensitivity as 10 and 11 mm zones of inhibition were recorded with leaf extracts, respectively. However, flower extracts exhibited similar zone of inhibition (9 mm) against both *E. aerogenes* and *S. aureus*. Pratima and Sundar<sup>26</sup> has reported high activity of the petroleum ether leaf extract of *Digera muricata* L. against *Vibrio cholerae*.

The antibacterial activities of Chloroform extracts of leaf and flower against *B. cereus*, *E. aerogenes* and *S. aureus* are shown in figures 3 a, b and c. Zone diameters of 7 mm and 10 mm were observed against *B. cereus* and *E. aerogenes* by chloroform leaf extract while, *S. aureus* was found to be insensitive at high dose of extract. Whereas,

chloroform flower extract showed inhibition zone of 11 mm against both *B. cereus* and *E. aerogenes* and 12 mm zone against *S. aureus*. Chloroform stem and root extract of *Andrographis paniculata* (Acanthaceae) was found effective against *Staphylococcus aureus*, *Bacillus subtilis* and *E.coli*.<sup>27</sup>

The methanolic extracts of leaf showed considerably high activity against *B. cereus*, *E. aerogenes* and *S. aureus* as 13, 10 and 14 mm zones of inhibition, respectively. However, methanol flower extract exhibited 7, 8 and 9 mm inhibition zone against *B. cereus*, *E. aerogenes* and *S. aureus*, respectively (Figures 4a, b and c). The sensitivity of the test bacteria could be due to the presence of polyphenolic compounds such as flavonoids, tannins and most other reported bioactive compounds that are generally soluble in polar solvents such as methanol.<sup>28</sup> Ali et al<sup>29</sup> have also reported alkyl esters, sterols, flavonoids and pentacyclic triterpenes as the major components of *Tridax procumbens*. Methanol leaf extracts was selected for MIC and MBC assay as it exhibited maximum antibacterial potential (Table 2). Methanol leaf extract was found to be highly active against both *B. cereus* and *S. aureus* with MIC of 0.31 mg/ml and MBC of 1.25 mg/ml. However, *E. aerogenes* comparatively showed higher MIC (0.62 mg/ml) and MBC (2.5 mg/ml). The differential sensitivity of the test bacteria towards the extracts might be due to the nature of the antimicrobial agents present in the extracts and their mode of action on the different test bacteria.<sup>30</sup>

**Table 1:** Preliminary qualitative evaluation of secondary metabolites in leaves and flower of *Vernonia cinerea*.

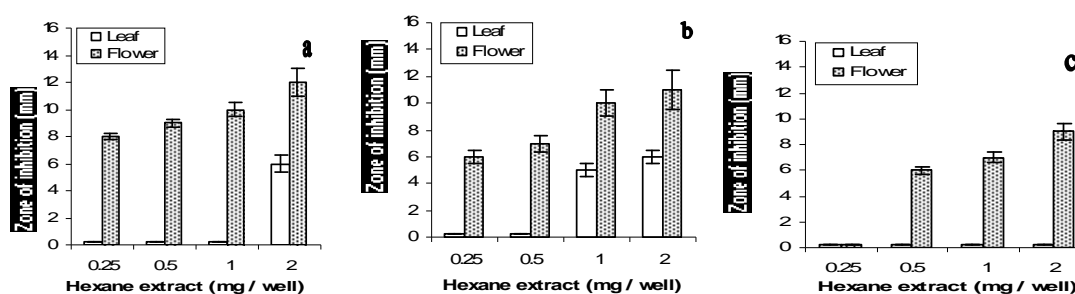
Plant part	Alkaloid	Flavonoid	Polyphenol	Saponin	Tannin
Leaf	++	+++	++	++	+
Flower	+	+++	++	+++	+

Note: + Slightly present, ++ Moderately present, +++ Highly present

**Table 2:** Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of leaf extracts of *Vernonia cinerea*

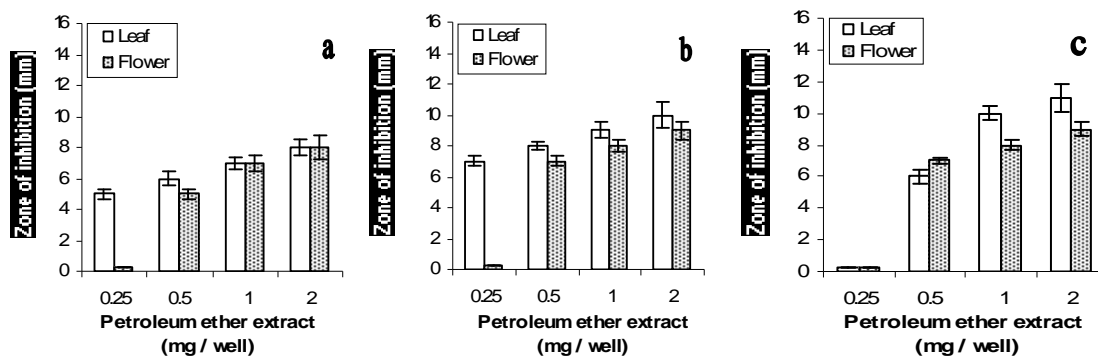
Bacteria	Methanol extract	
	MIC (mg / ml)	MBC (mg / ml)
<i>Bacillus cereus</i>	0.31	1.25
<i>Staphylococcus aureus</i>	0.31	1.25
<i>Enterobacter aerogenes</i>	0.62	2.5

The data refer to mean value of three replicates  $\pm$  SE.

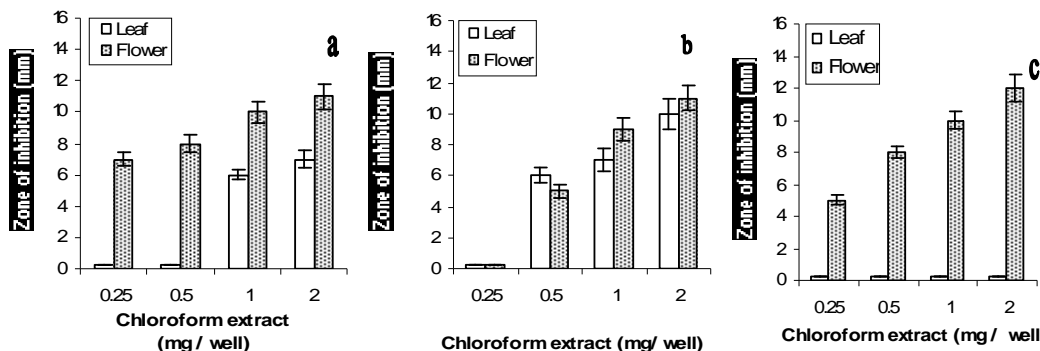


**Figure 1:** Antibacterial activity of Hexane extracts of leaf and flower of *Vernonia cinerea* against *B. cereus* (a), *E. aerogenes* (b) and *S. aureus* (c). Values are means  $\pm$  SE ( $n = 3$ ).

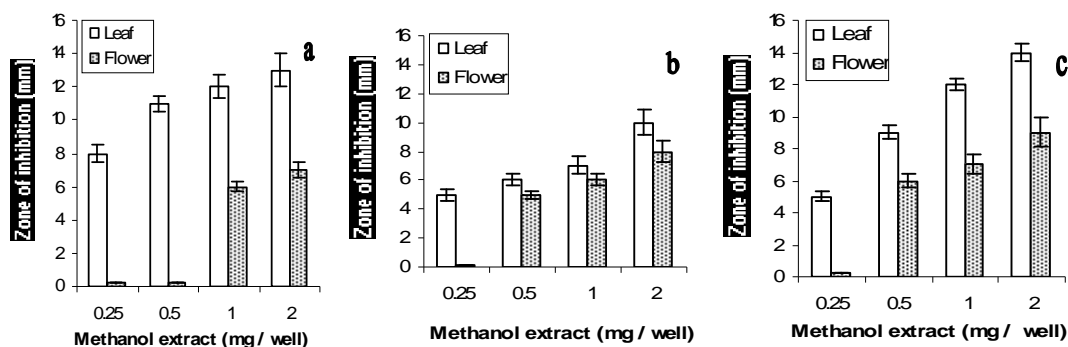




**Figure 2:** Antibacterial activity of Petroleum Ether extracts of leaf and flower of *Vernonia cinerea* against *B. cereus* (a), *E. aerogenes* (b) and *S. aureus* (c). Values are means  $\pm$  SE (n = 3).



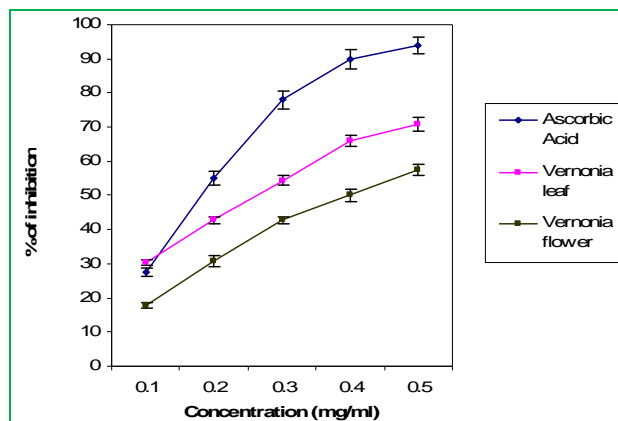
**Figure 3:** Antibacterial activity of Chloroform extracts of leaf and flower of *Vernonia cinerea* against *B. cereus* (a) and *E. aerogenes* (b) and *S. aureus* (c). Values are means  $\pm$  SE (n = 3).



**Figure 4:** Antibacterial activity of methanol extracts of leaf and flower of *Vernonia cinerea* against *B. cereus* (a), *E. aerogenes* (b) and *S. aureus* (c). Values are means  $\pm$  SE (n = 3).

Methanol extract of leaf showed higher antioxidant activity as compared to the flower extract (Figure 5). This result was in accordance with the earlier work conducted on leaf extract of *S. pinnata* by Mai et al<sup>31</sup>. Gowri and Vasantha<sup>32</sup> have also reported the antioxidant potential of leaf and flower extract of *Sesbania grandiflora*. Recently, many natural antioxidants have been isolated from different plant materials.<sup>33, 34</sup>

The presence of wide range of these phytochemical constituents indicates that *Vernonia cinerea* could serve as lead plant source for the development of novel agents for various pathological disorders. More work is currently ongoing in the author's laboratory to isolate and purify some of bioactive principles responsible for the antimicrobial and other biological potential.



**Figure 5:** Effects of leaf and flower methanol extracts of *Vernonia cinerea* and the standard ascorbic acid on the scavenging of DPPH. The data represent the percentage DPPH scavenging. All data are expressed as mean  $\pm$  SE (n = 3).



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